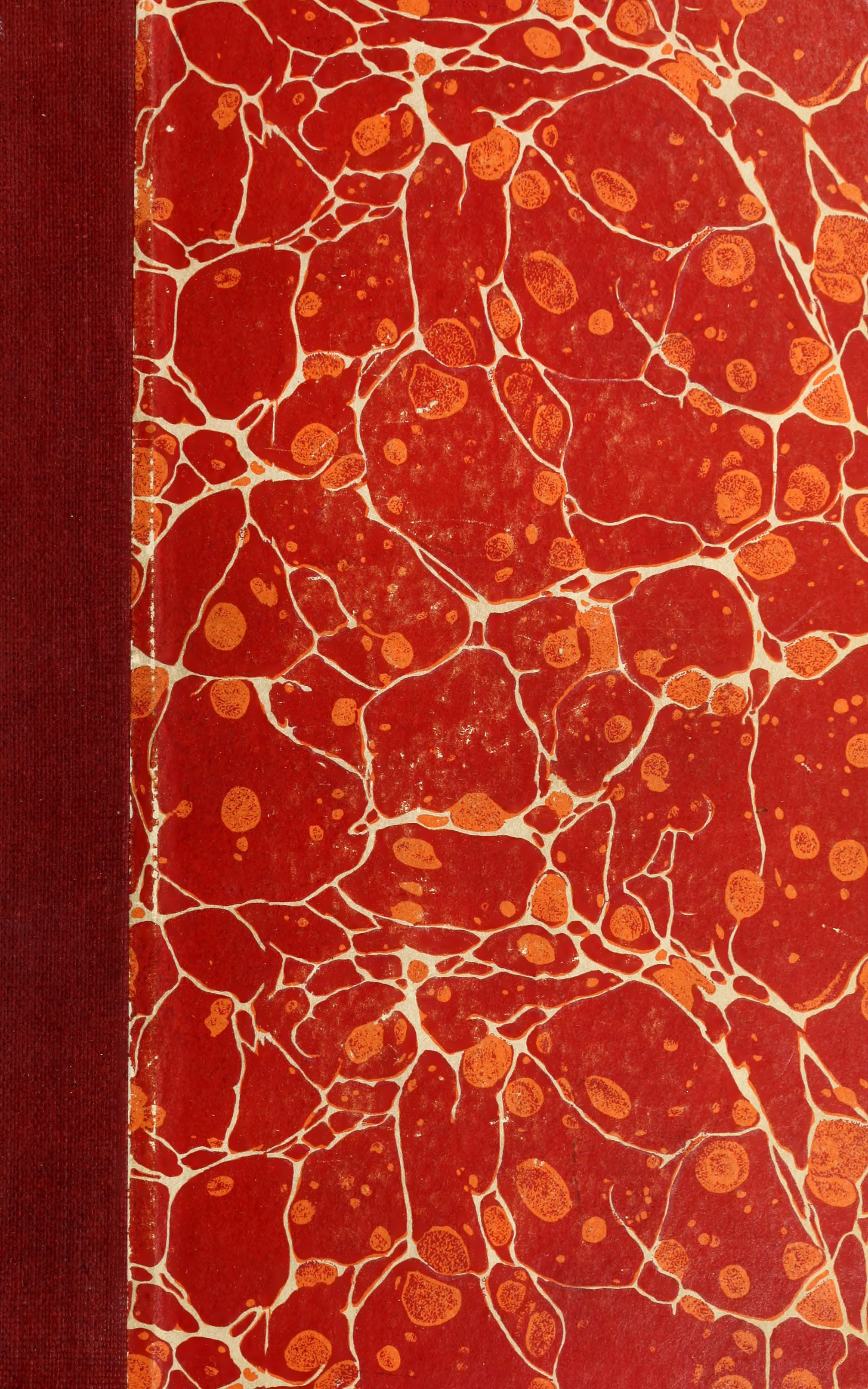

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RESEARCH STUDIES ON THE CURING OF LEAF TOBACCO.

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INTRODUCTION.

The term "curing," as applied to tobacco, is somewhat indefinite in meaning, being used sometimes to include the separate operations of barn curing, fermentation, and aging, or afterfermentation, while the farmer usually restricts the term to the process of drying the ripe leaf in a specially constructed barn and under such conditions as will develop the desired properties or qualities. In the present article the term is used in the last-named more restricted sense, so that we have only to consider curing as far as it proceeds in the curing barn.

The methods now in vogue in barn curing are almost entirely empirical, being the result of practical experience extending through several generations, and in general are based more or less on rule-of-thumb procedures, without sufficient flexibility to meet changing conditions and requirements. The barn curing of tobacco has not received the attention from investigators that has been given the subsequent process of fermentation, and such investigations as have been made relate mostly to certain special phases of the subject.

There are two general methods of harvesting tobacco and arranging it in the barn which materially affect the results obtained in curing.[1] In the one case the leaves are picked from the stalk as they mature or "ripen" and are arranged on strings or sticks suitable for hanging in the curing shed, this method being popularly spoken of as priming. In the other method the leaves are not removed from the stalks, but the latter are cut off near the ground and suspended in an inverted manner in the barn. There is an important modification of the method of harvesting and curing the leaves on the stalk in that, prior to being severed near the base, the stalk is split longitudinally from the top down the greater part of its length and when cut is placed astride a stick for hanging in the barn.

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[1] For details of these methods consult Garner, W. W., Principles and practical methods of curing tobacco, U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin 143, 54 p., 10 fig., 1909.

Note.—This bulletin gives the results of a study of the physiological changes occurring in curing tobacco in the barn and is of special interest to those concerned with the improvement of tobacco curing.

NATURE OF THE CURING PROCESS.

Observant growers well know that tobacco leaves which have been killed by freezing or by bruising do not cure normally, and a leaf which is quickly dried by heat does not possess the properties of the cured leaf. Moreover, if the fresh leaf is subjected for a few minutes to the action of protoplasmic poisons, such as formaldehyde or chloroform, it will not cure properly. It is quite evident, therefore, that curing is a life process and thus differs fundamentally from the subsequent fermentation, which takes place after the death of the leaf cells. That curing is essentially a life process can also be readily shown by chemical analysis, as is brought out in later paragraphs.

Curing materially changes the physical and chemical properties of the tobacco leaf, more particularly such properties or qualities as the capacity to hold fire, the color, the texture, and the elasticity. It does not develop the aroma, however. With respect to the development of some of the above-mentioned properties of the leaf, fermentation may be regarded as supplementary to curing; but, as we hope to show more fully in a later publication, some of the important changes in composition involved in curing are not continued in the fermentation, so that incomplete curing can not be fully corrected in the subsequent fermentation.

As will be shown later, curing involves principally the two familiar physiological processes of respiration and of the translocation of mobile nutrients. Some investigators have preferred to regard the respiration phenomena as essentially pathological in character, and this view seems logically correct. The stalk and its leaves or the leaves alone, as the case may be, after harvesting are deprived of the water solution from the root system and for the most part are deprived of sunlight. Since respiration continues, the living cells of the leaf are subjected to a process of starvation, and the degree to which the starvation proceeds is largely dependent on the rate of drying. As a matter of fact, however, the general character of the changes in composition during the curing, due to respiration, is very much the same as in normal respiration. As would be expected, the prevailing temperature and humidity play an important rôle in the process. The other important phenomenon, translocation, involves the movement of soluble materials from the leaf web, through the veins, into the midrib, and, if the leaf is attached to the stalk, thence into the latter. With one or two exceptions, investigators have overlooked the importance of translocation when the leaf is cured on the stalk.

It is, of course, well known that during the growth and development of the plant there is a movement of the products of photosynthesis, rendered soluble by enzyms when necessary, from the more mature leaf through the stalk to the younger growing parts. Essentially the same thing takes place after the cut plant has been placed in the barn, except that the materials transported are derived wholly from the surplus food supply of the leaf. While the leaf web perishes in a few days, the midrib remains alive for longer periods and the stalk may remain perfectly green for several weeks. Secondary shoots, or suckers, developing in the leaf axils, may be found growing at the end of several weeks, and the necessary food supply for the stalk and the suckers is derived from the leaf surplus. An interesting confirmation of this phenomenon is found in the case of excessive moisture prevailing in the barn during the later stages of curing, a condition which leads to the decay of the leaf known as pole-sweat. The excessive moisture causes the dying leaf cells to retain more water, thus facilitating the more complete translocation of food materials into the stalk. Under these circumstances the so-called absciss-layer is formed at the juncture of the midrib with the stalk, so that the leaf is cast off as a useless appendage. Under normal curing conditions the leaves remain firmly attached to the stalk.

LOSS IN WEIGHT OF DRY MATTER IN AIR CURING WHEN THE LEAF IS PRIMED.

The first problem met with in a physiological study of curing is the total loss in dry weight which occurs in the process. So far as we know, no accurate determinations have been previously made on this point, at least in this country. Because of the important rôle of translocation in curing, the loss in weight depends largely on the method used in harvesting the tobacco. So far as it relates to loss in weight of the whole leaf, translocation can play no part when the leaf is separated from the stalk in harvesting.

Our experiments cover the crop years of 1908 to 1911, inclusive, a period of four years, and were confined to the cigar-wrapper-leaf section of Connecticut, the experimental material all being obtained from the farm of W. S. Pinney, Suffield, Conn. The types of tobacco included in the experiments are the Havana Seed, the Halladay, and a so-called John Williams broadleaf. The Havana seed is one of the old standard types of domestic cigar-wrapper leaf and the Halladay is a new type developed from a cross of Havana seed on Sumatra. The history of the John Williams type of broadleaf is not known, but it differs decidedly from the ordinary broadleaf, and its habits of growth strongly suggest its origin from a cross of the latter on Cuban.

In gathering the experimental material each year, a sufficient number of representative plants was selected to give about 200 ripe leaves near the bases of the plants, six or eight leaves being taken from each plant. The leaves were taken in pairs, each alternate leaf being placed in one lot and the other leaves in a second lot, the aim being to have each leaf in the one lot represented by a duplicate in the second lot. For the success of the experiment it is essential that the total dry weight and also the average composition of the two lots of leaves be practically the same. The detailed examination of the material gathered in the manner described indicates that these requirements have been satisfactorily met. As promptly as possible after harvesting the leaves the midribs were completely removed from the lot which was used to obtain the original dry weight. This was done to prevent any flow of cell sap between the leaf web and the midrib during the drying. The split leaves as well as the midribs were at once placed in a large drying oven and maintained at a temperature of about 80° C. until all were completely dry. The temperature employed rapidly kills the protoplasm, and comparative tests with other methods of killing, such as plunging in boiling absolute alcohol, showed that the respiration changes during the drying were too small to be of significance for our purposes. (See p. 35.) The dry weights of the leaf web and the midribs were obtained and the material preserved for analysis. The second lot of leaves was placed in the barn for curing in the usual way without removing the midribs. When the cured leaves were ready for examination the leaf web was removed from the midrib, the dry weights obtained, and the material preserved for analysis.

As has been stated, observations were made for a period of four consecutive years of the loss of dry matter in curing when the leaves are harvested by picking them from the stalk. The detailed results of these experiments are shown in section A of Table I (p. 5). In the case of the 1908 material the curing was stopped before the midribs had cured, but the curing of the leaf web was practically complete. In 1910 a special test of partial curing was made (Table I, fourth column) and in this case the curing was stopped while the larger veins and midribs were still uncured. In 1911 a test was made of the effect of artificial heat on the curing.

TABLE I.—*Loss in dry weight in air curing tobacco leaves: (A) by the priming method, (B) by curing on the stalk.*

Tobacco leaves.	A.—Leaves cured by the priming method.								
	Havana Seed; 1908 (partially cured).			Halladay; 1909.			Halladay; 1910.		
	Leaf web.	Stems.	Whole leaf.	Leaf web.	Stems.	Whole leaf.	Leaf web.	Stems.	Whole leaf.
Weight of 100 dry leaves:									
Uncured..........grams..	409.5	155.1	564.6	335.2	117.2	452.4	294.8	121.4	416.2
Cured..............do....	329.4	157.5	486.9	306	98	404	256.4	98.6	355
Loss of weight in curing, per cent......................	19.6	[1]−1.6	13.8	8.7	16.4	10.9	13	18.8	14.7
Leaves:									
Uncured.......per cent..	72.5	27.5	100	74.1	25.9	100	70.8	29.2	100
Cured..............do....	67.6	32.4	100	75.7	24.3	100	72.2	27.8	100
Weight of pure ash in 100 leaves:									
Uncured..........grams..	44.9	26.9	71.9	51.2	26.7	77.9	38.4	23.3	61.7
Cured..............do....	43.9	26.3	70.2	55.3	25.2	80.5	41.4	22.2	63.6
Apparent gain or loss of ash in curing........per cent..	−.24	−.38	−.29	+1.20	−1.28	+.57	+1.02	−.91	+.45
Loss of organic matter in curing[2].............per cent..	19.6	[1]−1.6	13.8	9.6	14.3	10.9	13.6	17.3	14.7

Tobacco leaves.	A.—Leaves cured by the priming method.								
	Halladay; 1910 (partially cured).			John Williams broadleaf.					
				1911-A (artificial heat applied).			1911-B (no artificial heat applied).		
	Leaf web.	Stems.	Whole leaf.	Leaf web.	Stems.	Whole leaf.	Leaf web.	Stems.	Whole leaf.
Weight of 100 dry leaves:									
Uncured.........grams..	269.3	120.7	390	402	114.8	516.8	402	114	516
Cured..............do....	234.3	108.3	342.6	356.8	87.6	444.4	356.5	87.8	444.3
Loss of weight in curing, per cent......................	13	10.3	12.2	11.2	23.7	14	11.3	23	13.9
Leaves:									
Uncured.......per cent..	69.1	30.9	100	77.8	22.2	100	77.9	22.1	100
Cured..............do....	68.4	31.6	100	80.3	19.7	100	80.4	19.6	100
Weight of pure ash in 100 leaves:									
Uncured..........grams..	34	22.3	56.4	48.9	23.8	72.7	48.3	24.2	72.5
Cured..............do....	36.3	21.4	57.6	53.4	21	74.4	52.2	20.9	73
Apparent gain or loss of ash in curing........per cent..	+.85	−.74	+.31	+1.12	−2.43	+.33	+.97	−2.89	+.11
Loss of organic matter in curing............per cent..	13.5	9.1	12.2	12.1	19.1	14	12.2	19.9	13.9

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[1] Represents gain.

[2] These figures are arrived at by deducting from the total loss in weight the apparent translocation of ash between leaf tissue and stem. See page 6.

TABLE I.—*Loss in dry weight in air curing tobacco leaves: (A) by the priming method, (B) by curing on the stalk*—Continued.

Tobacco leaves.	B.—Leaves cured on stalk.								
	Havana Seed; 1908 (partially cured).			Halladay; 1909.			Halladay; 1910.		
	Leaf web.	Stems.	Whole leaf.	Leaf web.	Stems.	Whole leaf.	Leaf web.	Stems.	Whole leaf.
Weight of 100 dry leaves:									
Uncured.........grams..	437.1	165.9	603	342.9	145.4	488.3	297.6	123.1	420.7
Cured..............do....	312.4	134	446.4	238.1	104.6	342.7	230	86.7	316.7
Loss of weight in curing, per cent........................	28.6	19.2	26	30.6	28.1	29.8	22.7	29.6	24.7
Leaves:									
Uncured.......per cent..	72.5	27.5	100	70.2	29.8	100	70.7	29.3	100
Cured..............do....	70	30	100	69.5	30.5	100	72.6	27.4	100
Weight of pure ash in 100 leaves:									
Uncured.........grams..	45.3	28.4	73.7	42.3	27.9	70.1	38.6	23.2	61.9
Cured.............do....	41.4	26.4	67.8	40.3	23.1	63.4	37.3	18.6	55.9
Loss of ash in curing, per cent.	.89	1.20	.98	.59	3.31	1.38	.43	3.74	1.42
Loss of organic matter in curing...........per cent..	27.7	18	25	30	24.8	28.4	22.3	25.9	23.3

The content of pure ash is of special interest as serving as a check on the original uniformity in size and composition of the uncured and cured leaves of each series, since there could be no actual loss of ash from the whole leaf. The differences found between the uncured and the cured leaves are so small as to be negligible, and they furnish strong evidence of the uniformity in size and composition of the original duplicate samples. We are safe, therefore, within very narrow limits, in regarding the observed differences in weight between the uncured and cured leaves as representing the true loss in weight of dry matter during the curing process.

Regarding the total loss in weight of the whole leaf, it will be seen that the values ranged from about 11 to about 14.5 per cent when the curing was completed. In the case of the 1909 sample of Halladay tobacco the loss is undoubtedly below normal, and the low value for the whole leaf is due mainly to the slight loss in weight of the leaf web. It is not known to what extent unfavorable weather conditions during the curing may have influenced the result, but by reference to Table IV, section A (p. 18), it will be seen that the sample was very low in starch content, and this constituent is the most important of all as regards loss in weight in curing. (See also p. 31.) Disregarding this sample, the loss in weight in the experiments is surprisingly uniform in view of the differences in types of leaf and the varying weather conditions during the curing. In the two cases where the curing was interrupted before it was complete, the loss in weight of the whole leaf was nearly as great as in complete curing, showing that the principal changes in composition to which the loss in weight is due proceed rapidly. In the case of the Havana Seed sample (1908) the loss would undoubtedly have been considerably greater had the stems been permitted to cure, as is to be expected, because of the relatively high starch content in this sample. For the types of leaf considered, the average loss in weight in dry matter during the curing of the picked leaves may be placed at about 12 to 15 per cent.

The data presented in Table I, section A, relate only to types of tobacco adapted to and grown under conditions favorable to the production of cigar-wrapper leaf, which is relatively very thin and light. This class of tobacco is harvested in a less ripe condition than are other classes of leaf, so that a lower content of starch is to be expected in the leaves when harvested. For these reasons the loss in dry weight in curing is greater in other classes of tobacco, and in the case of the export types losses in weight as high as 38 to 40 per cent have been observed. The loss in weight in curing varies with the variety or type grown, the conditions under which the tobacco is grown, the degree of ripeness when it is harvested, and also with the conditions under which the curing takes place. The effects of these factors on the loss in weight are due to their influence on the composition of the leaf before or after the curing, particularly on the content of starch.

LOSS IN WEIGHT OF DRY MATTER IN AIR CURING WHEN THE LEAF IS CURED ON THE STALK.

The experiments relating to the loss in weight when the leaves are cured on the stalk cover the crop years of 1908 to 1910, inclusive, a period of three years, and the experimental material was obtained from the same fields as was that used in the preceding experiments. The stalks of the plants used were not split in harvesting, but were hung in the barn in the usual manner for curing cigar tobaccos. Every effort was made to have the experimental material directly comparable with that used in the preceding experiments for the same years. Plants were selected which were as nearly as possible like those used for the experiments in priming. Beginning near the base of the plant each alternate leaf was picked off, taking three or four leaves from each plant and leaving all of the remaining leaves intact. The stalks were then harvested and placed in the barn for curing. The midribs were promptly removed from the picked leaves and both the leaf web and the midribs completely dried, as in the previous experiment. The dry weights of the leaf parts were recorded and the material preserved for analysis. When the leaves attached to the stalks were cured the weights of the leaf web and midribs were likewise obtained and the material kept for further examination. As in the case of the experiments in priming, the leaves were removed from the stalks in the 1908 experiment before the midribs were fully cured. With the possible exception of the 1909 material, the ash content and the ratio of leaf web to rib in the uncured leaves, as well as the detailed analyses later reported, show conclusively that the original samples used for stalk curing were very similar to the corresponding samples used in curing by priming.

The results of the three-year experiment are set forth in detail in section B of Table I. It will be seen at a glance that the loss in weight of the whole leaf in curing on the stalk is far greater than when the leaves are separated from the stalks. The figures run from about 25 to 30 per cent, or approximately double those for curing the picked leaves. In the 1908 sample the total loss in weight would have been somewhat greater had the stems been given sufficient time for complete curing. The loss in weight in stalk curing is greater in both leaf web and rib than when the picked leaves are cured. It will be seen that the apparent loss in pure ash is positive and marked in all cases in both the leaf web and the ribs, showing conclusively that a portion of the ash passes into the stalk during the curing.

EFFECT OF SPLITTING THE STALK ON THE LOSS OF WEIGHT IN AIR CURING.

In some tobacco districts the stalk is split longitudinally from the top down the greater part of its length at the time the plant is harvested. Under these conditions the stalk can not remain alive in the barn as long as when it is merely severed from the rootstock, and it is to be expected that the phenomenon of translocation would be less important. A special experiment was carried out to secure information on this point.

Two similar lots of 10 plants each were selected, and the stalks of one lot were split in the manner followed by growers. Each alternate leaf was picked from the plants in both lots, and the two lots of leaves thus obtained were cured separately. The leaves remaining on the stalks were cured under the same conditions as the primed leaves. When the curing was complete the dry weights were obtained as in the preceding experiments. At that time the unsplit stalks were still green, while the split stalks had largely dried out.

TABLE II.—*Loss of dry weight in air curing as affected by splitting the stalk in harvesting.*

Air-cured leaves.	Stalks split in harvesting.			Stalks not split in harvesting.		
	Leaf web.	Stems.	Whole leaf.	Leaf web.	Stems.	Whole leaf.
Weight of 50 leaves:						
Cured on stalk....................grams..	177.9	70.1	248	176.5	68.3	244.8
Cured after picking..............do....	219.5	81.1	300.6	227	84.6	311.6
Difference in loss of weight between stalk curing and curing picked leaves, per cent....................................	18.9	13.6	17.4	22.2	19.3	21.4
Leaves consisted of:						
Crued on stalk...............per cent..	71.8	28.2	....	72.1	27.9	....
Crued by picking................do....	73	27	....	72.8	27.2	....

The results of the experiment are shown in Table II. While the actual number of leaves in each lot was 58, the results are calculated on a basis of 50 leaves for convenience in comparing with the other experiments. The experiment, of course, does not show the total loss in weight in curing in any case, but other experiments with similar material from the same field indicate that the loss in weight of the picked leaves must have been more than 20 per cent. The type of tobacco and the stage of ripeness account in all cases for the larger losses in weight than were obtained with the cigar types. Since only a single experiment was made and a rather small number of leaves used, the results can be taken as only approximately correct. These results indicate that when the stalk is split in harvesting, the loss in weight in curing is less than when the stalk is not split, but, nevertheless, the loss is much greater than when the leaves are picked from the stalk.

COMPOSITION OF CIGAR-WRAPPER LEAF BEFORE AND AFTER CURING.

The material obtained in connection with the data presented in Table I was subjected to analysis to ascertain the nature of the changes in composition which take place in air curing and to which the losses in dry weight are due. Because of the variations in the composition of the leaf at the time of harvesting, it is obviously essential that the material chosen for study shall be obtained under such conditions as will insure original uniformity in the composition of the uncured and cured samples. That this has been very closely attained in the above-mentioned material is shown in the values obtained for those constituents which undergo no change in the curing.

LEAF HARVESTED BY PRIMING.

The samples[1] used in these experiments cover the curing seasons of 1908 to 1911, inclusive, and include cases in which the curing was not fully completed. Although the midrib is never removed prior to curing, it has little or no value in manufacturing and, moreover, differs decidedly in composition from the leaf web, so that for present purposes it is necessary to consider the two leaf parts separately. The cured leaf always contains more or less water, depending on the type in question and on atmospheric conditions, but to simplify matters all results have been calculated to a water-free basis.

PREPARATION OF MATERIAL AND METHODS OF ANALYSIS EMPLOYED.

To pulverize the material for analysis, the leaf web was simply passed through a 60-mesh wire sieve. The stems were prepared by grinding to a fine powder in an iron mortar. The water content of the sample was determined by drying over sulphuric acid, as recommended by

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[1] For details as to the methods followed in selecting and gathering the samples, the type of leaf used, etc., see p. 3.

Kissling. The pure ash, the carbohydrates, including starch, reducing sugars, pentosans, and crude fiber, and the several nitrogenous constituents were determined by the following methods:

Pure ash.—The tobacco was incinerated in the usual manner in a platinum dish with a cover, care being taken to avoid fusing the ash. The pure ash is obtained by correcting for carbon dioxid, carbon, and sand.

Starch.—Rather than attempt to determine starch directly by the diastase method it was preferred to obtain the total of the carbohydrates hydrolyzed by hydrochloric acid, using the official method, and to determine also the pentosans. It is thought that for purely comparative results this method is satisfactory, and nearly all previous analyses of tobacco for starch which the writers have seen have been based on this method of the direct hydrolysis with acid, but usually without determining the pentosans.

Pentosans.—The determinations were made by the official method, the phloroglucin being calculated to pentosan by Kröber's tables.

Reducing sugars.—Since nicotine, even in a moderately concentrated solution, exerts a reducing action on Fehling's solution, it is removed before preparing the solution for the determination of reducing sugars. This is done by moistening 5 grams of the tobacco with 5 c. c. of a 5 per cent solution of caustic potash in absolute alcohol and digesting the mixture in a flask with 100 c. c. of absolute ether. The reducing sugar is then extracted with 60 per cent alcohol, the alcohol removed by evaporation, the water solution made up to volume, clarified with normal lead acetate, and the sugar determined as glucose by the Allihn method.

Crude fiber.—The official method, as modified by Sweeney and by Kennedy, was employed.

Nonvolatile organic acids.—The method of Kissling[1] was followed for the determination of oxalic, citric, and malic acids. We have found it necessary, however, to increase by at least 25 per cent the quantity of sulphuric acid recommended by Kissling to be added to the tobacco, to insure the liberation of all the organic acids. This was particularly true of the cured samples, from which only very small portions of the oxalic acid could be extracted when only 10 grams of a 20 per cent sulphuric-acid solution were added to 10 grams of the tobacco.

Protein nitrogen.—Tests having shown that the method of Mohr[2] gives excellent results for the purpose in view, it was followed in preference to the official (Stutzer) method. In this process the tobacco is simply boiled with a dilute acetic-acid solution, filtered cold, and, after washing with hot water acidified with acetic acid, the nitrogen in the residue is determined by the Kjeldahl method. Comparative tests of cured and uncured samples of tobacco show that the Stutzer method regularly gives results about 0.2 per cent higher than the Mohr method. The protein is estimated by multiplying the nitrogen obtained as indicated by the usual factor, 6.25.

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[1] Kissling, Richard. Beiträge zur Chemie des Tabaks. Zur Tabakanalyse. Chemiker-Zeitung, Jahrg. 28, No. 66, p. 775–776, 1904.

[2] Mohr, E. C. J. Gepflückter und am Stamme getrockneter Tabak. Die Landwirtschaftlichen Versuchs-Stationen, Bd. 59, p. 274, 1903.

Nicotine.—This constituent was determined by the method described in Part VII of Bureau of Plant Industry Bulletin No. 102, entitled "A New Method for the Determination of Nicotine in Tobacco," tests having shown that this process gives as accurate comparative results as does the well-known Kissling method.

Nitric acid.—The method of Kissling [1] was followed in this determination.

Ammonia.—This constituent was determined in the filtrate from the protein nitrogen by the Folin method as modified by Pennington and Greenlee.[2] Under the conditions, only traces of nicotine are carried over into the receiver by the air current. However, in our determinations the results were corrected by running check solutions containing the same quantities of nicotine as were known to be contained in the solutions from the tobacco samples. The temperature, rate of air current, etc., were kept as uniform as possible in making the determinations.

Amid and amido nitrogen.—This value was obtained by taking the difference between the total nitrogen and the sum of the protein, nicotine, nitrate, and ammonia nitrogen. The figure obtained was multiplied by 4.8 (the approximate factor for asparagin) for amid and amido compounds.

Total nitrogen.—The official Gunning method, as modified for nitrates, was used.

RESULTS OBTAINED IN THE FOUR-YEAR EXPERIMENT.

In section A of Table III (p. 12) will be found the results of the analyses of the leaf samples previously described. The leaf web and the stems or midribs were, of course, analyzed separately, and the results for the whole leaf were calculated from the ratio of leaf web to stem in each case. As a matter of convenience, the ripe, uncured leaf is designated in the table as "green."

The content of pure ash of the cured leaves is higher than that of the uncured leaves in proportion to the loss in dry weight in curing, since there can be no change in the quantity of ash during the curing. While the green leaves contain considerable but quite variable quantities of starch, the cured leaves contain practically none, as shown by

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[1] Kissling, Richard. Handbuch der Tabakkunde, des Tabakbaues und der Tabakfabrikation. Aufl. 2, Berlin, 1905, p. 78.

[2] Pennington, M. E., and Greenlee, A. D. An application of the Folin method to the determination of the ammoniacal nitrogen in meat. Journal, American Chemical Society, v. 32, p. 561-568, 1 fig., 1910.

TABLE III.—*Composition of cigar-wrapper leaf before and after curing.*

A.—LEAVES CURED BY THE PRIMING METHOD.

Constituents.	Havana Seed; 1908 (partially cured).						Halladay; 1909.						Halladay; 1910.					
	Leaf web.		Stems.		Whole leaf.		Leaf web.		Stems.		Whole leaf.		Leaf web.		Stems.		Whole leaf.	
	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.
Pure ash	10.97	13.33	17.36	16.70	12.72	14.42	15.28	18.06	22.78	25.69	17.23	19.89	13.03	16.14	19.15	22.55	14.82	17.92
Starch [1]	16.38	6.91	10.82	8.76	14.88	7.41	5.77	4.78	9.94	8.26	6.85	5.62	7.53	5.22	7.76	7.63	7.60	5.89
Sugars	3.32	3.46	10.40	11.90	5.27	6.19	1.33	.60	9.78	.56	3.52	.59	1.97	0	10.88	0	4.57	0
Pentosans	4.21	5.36	7.91	7.69	5.22	6.10	4.84	5.34	7.48	7.67	5.53	5.90	....	....	....	....	....	....
Cellulose (crude fiber)	8.50	10.22	13.12	13.22	9.77	11.19	....	....	....	....	....	....	....	....	....	....	....	....
Oxalic acid	1.54	2.18	1.27	1.06	1.47	1.82	....	....	....	....	....	....	....	....	....	....	....	....
Citric acid	5.88	10.87	3.54	2.77	5.24	8.25	....	....	....	....	....	....	....	....	....	....	....	....
Malic acid	10.27	7.98	10.49	10.50	10.33	8.80	....	....	....	....	....	....	....	....	....	....	....	....
Protein [2]	11.37	7.00	3.87	3.75	9.31	5.95	16.06	9.87	4.25	4.87	12.99	8.69	21.20	11.44	4.50	5.44	16.38	9.75
Nicotine	4.60	4.74	.51	.50	3.48	3.60	2.58	2.53	.38	.42	2.02	2.06	1.77	1.82	.17	.22	1.30	1.16
Nitric acid (N_2O_5)	.12	.08	1.04	1.04	.37	.33	2.47	2.23	12.42	11.77	5.04	4.54	....	....	....	....	....	....
Ammonia	.04	.18	0	.03	.03	.14	....	....	....	....	....	....	....	....	....	....	....	....
Amid and amido compounds.[3]	3.22	6.38	.12	3.89	2.64	5.62	1.92	6.67	0	1.68	1.42	5.46	....	....	....	....	....	....
Total nitrogen	3.32	3.44	1.22	1.80	2.75	2.91	4.05	3.99	3.66	4.27	3.95	4.07	4.61	4.86	2.89	3.80	4.11	4.57
Undetermined [4]	23.79	25.83	27.46	25.88	24.49	26.42	....	....	....	....	....	....	....	....	....	....	....	....

Constituents.	Halladay, 1910 (partially cured).						John Williams broadleaf.											
							1911-A (artificial heat applied).						1911-B (no artificial heat applied).					
	Leaf web.		Stems.		Whole leaf.		Leaf web.		Stems.		Whole leaf.		Leaf web.		Stems.		Whole leaf.	
	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.
Pure ash	12.64	15.47	18.48	19.73	14.40	16.78	12.16	14.97	20.72	24.02	14.06	16.75	12.02	14.62	21.21	23.93	14.05	16.46
Starch[1]	7.74	5.02	7.87	7.44	7.78	5.79	7.54	4.93	9.41	8.30	7.96	5.59	5.86	5.07	10.60	9.93	6.91	6.03
Sugars							1.64	.27	8.40	.05	3.14	.23	1.30	.25	7.94	.06	2.77	.21
Pentosans							4.72	5.34	8.45	8.95	5.55	6.05	4.78	5.23	9.28	9.88	5.77	6.15
Cellulose (crude fiber)							7.82	8.94	13.87	17.03	9.16	10.53	10.98	12.09	14.46	18.18	11.75	13.28
Oxalic acid							2.19	2.79	1.15	1.30	1.96	2.50	2.32	2.80	1.36	1.55	2.11	2.55
Citric acid							8.52	13.83	2.32	4.45	7.14	11.98	9.52	15.20	2.46	5.13	7.96	13.21
Malic acid							6.89	5.17	5.87	3.81	6.66	4.90	7.28	4.51	5.96	2.55	6.99	4.12
Protein[2]	21.0	10.75					22.56	9.81	4.63	4.87	18.56	8.84	21.19	9.08	4.38	4.75	17.50	8.31
Nicotine							3.29	3.43	.58	.78	2.69	2.91	3.74	3.43	.67	.82	3.06	2.96
Nitric acid (N_2O_5)							1.07	1.54	8.62	9.30	2.75	2.95	.87	1.56	8.00	7.89	2.44	2.72
Ammonia							.07	.45	.05	.32	.07	.42	.03	.61	.04	.23	.03	.55
Amid and amido compounds[3]							3.60	12.53	0	3.17	2.80	10.68	4.32	10.70	0	2.06	3.37	8.99
Total nitrogen							5.27	5.53	3.03	4.25	4.77	5.32	5.18	5.19	2.84	3.41	4.66	4.84
Undetermined[4]							22.65	21.34	24.38	22.60	23.00	21.72	20.57	20.08	22.92	22.86	21.06	20.61

[1] Includes total carbohydrates hydrolyzed by dilute acid.

[2] Protein nitrogen × 6.25.

[3] Total nitrogen less nitrogen as protein, nicotine, nitric acid, and ammonia × 4.8. With two exceptions (1908) the sums of the protein, nicotine, nitrate, and ammonia nitrogen of the green stems exceed slightly the values obtained for total nitrogen. For the probable explanation, see text (p. 28).

[4] Pentosans and total nitrogen are not included in the calculation of this value.

TABLE III.—*Composition of cigar-wrapper leaf before and after curing*—Continued.

B.—LEAVES CURED ON THE STALK.

Constituents.	Havana Seed, 1908 (partially cured).						Halladay; 1909.						Halladay; 1910.					
	Leaf web.		Stems.		Whole leaf.		Leaf web.		Stems.		Whole leaf.		Leaf web.		Stems.		Whole leaf.	
	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.	Green.	Cured.
Pure ash	10.36	13.28	17.14	19.67	12.22	15.20	12.32	16.91	19.18	22.10	14.36	18.49	12.98	16.22	18.87	21.50	14.70	17.67
Starch[1]	16.97	6.56	10.64	10.52	15.23	7.75	8.22	7.59	12.41	9.29	9.47	8.13	6.14	6.27	8.23	7.78	6.75	6.68
Sugars	2.29	2.97	8.57	4.37	4.01	3.39	1.89	.64	9.77	.24	4.24	.52	1.02	0	8.69	0	3.26	0
Pentosans	4.45	5.81	8.04	9.43	5.44	6.90	4.61	6.59	8.54	8.65	5.78	7.30	....	....	....	....	....	....
Cellulose (crude fiber)	7.28	10.98	13.00	16.37	8.85	12.60	....	....	....	....	....	....	....	....	....	....	....	....
Oxalic acid	1.60	2.52	1.29	1.51	1.51	2.21	....	....	....	....	....	....	....	....	....	....	....	....
Citric acid	5.45	13.95	4.41	5.29	5.16	11.35	....	....	....	....	....	....	....	....	....	....	....	....
Malic acid	9.21	6.77	9.70	10.06	9.34	7.76	....	....	....	....	....	....	....	....	....	....	....	....
Protein[2]	13.12	7.25	3.87	3.81	10.56	6.19	22.40	16.06	4.63	4.25	17.06	12.50	20.00	11.13	4.44	4.31	15.44	9.26
Nicotine	4.11	5.21	.87	.55	3.21	3.71	2.17	2.32	.45	.45	1.66	1.74	1.53	1.89	.22	.17	1.15	1.42
Nitric acid (N_2O_5)	.11	.08	1.60	1.61	.52	.56	1.75	2.61	9.92	9.99	4.18	4.90	1.11	2.15	11.02	11.11	4.01	4.59
Ammonia	.07	.07	0	.03	.05	.06	....	....	....	....	....	....	....	....	....	....	....	....
Amid and amido compounds[3]	3.46	3.26	1.58	1.54	2.98	2.74	2.35	1.06	0	0	1.65	.74	7.25	7.68	0	.91	5.13	5.54
Total nitrogen	3.61	2.82	1.51	1.46	3.03	2.41	4.91	3.87	3.03	3.39	4.34	3.72	5.26	4.28	3.21	3.32	4.66	4.05
Undetermined[4]	25.97	26.96	27.33	22.67	26.36	26.38	....	....	....	....	....	....	....	....	....	....	....	....

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[1] Includes total carbohydrates hydrolyzed by dilute acid.

[2] Protein nitrogen × 6.25.

[3] Total nitrogen less nitrogen as protein, nicotine, nitric acid, and ammonia × 4.8. With two exceptions (1908) the sums of the protein, nicotine, nitrate, and ammonia nitrogen of the green stems exceed slightly the values obtained for total nitrogen. For the probable explanation, see text (p. 28).

[4] Pentosans and total nitrogen are not included in the calculation of this value.

the iodin test. Cigar-wrapper-leaf types contain less starch at the time of harvesting than other commercial types of leaf, because the former are harvested at a less mature stage and are produced under conditions less favorable to the accumulation of starch during the ripening period. Müller-Thurgau[1] has shown that in some cases fully ripe European-grown tobacco leaves may contain upward of 40 per cent of starch, and we have found this to be true of some samples of our export types. (Compare pp. 35 and 38).

One of the most marked physiological differences between the green and cured leaves is the content of protein insoluble in dilute acid. In all cases the protein content of the cured leaves is much less than that of the uncured leaves.

The content of nitric acid in the green and in the cured leaves is about the same. The green leaves at most contain only traces of ammonia, while the cured leaves contain considerable quantities. The cured leaves contain relatively much larger quantities of amid and amido compounds than the green leaves. The relative content of total nitrogen is somewhat less in the green than in the cured leaves.

LEAF HARVESTED ON THE STALK.

The method followed in the preparation of the material and the methods of analysis employed were the same as for the leaves harvested by priming. The original material was chosen with a view to obtaining samples which would be duplicates, essentially, of those used in the preceding experiments, in order that the stalk-cured leaves might be strictly comparable with those cured after being picked from the stalk. It is, of course, a simple matter to check by analysis the original uniformity of the corresponding lots of the green leaves in each case, since these were detached from the stalks, the leaf web separated from the midribs, and the leaf parts quickly dried. This can not be done so readily, however, with the two lots of cured leaves, because of the fact that when the leaves are cured on the stalk there is a well-defined movement of certain constituents from the leaf into the stalk, causing an additional loss in weight, which can only be determined by direct experiments, as reported in Table I, which require that the green and the cured leaves shall possess the same original weight.

RESULTS OBTAINED IN THE THREE-YEAR EXPERIMENT.

The comparative composition of cigar-wrapper leaf cured on the stalk and of the corresponding uncured leaf is shown in section B of Table III. As in the primed leaves, the ash content of the whole leaf is higher in the cured than in the uncured leaves. The cured leaves are again practically free from starch and reducing sugars, except where the curing was incomplete. The difference as regards protein is similar to those noted in the cured and uncured leaves in the preceding experiments. The differences with reference to amid and amido compounds are somewhat variable, but it is evident that the cured leaf does not contain appreciably larger quantities of these constituents, relatively, than the green leaf, and the same is true as to ammonia. It is clear that the cured leaves contain considerably less total nitrogen than the green leaves.

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[1] Müller, Hermann (*Thurgau*). Ueber das Verhalten von Stärke und Zucker in reifenden und trocknenden Tabaksblättern. Landwirtschaftliche Jahrbücher, Bd. 14, p. 485–512, 1885.

Analyses of cured samples of our more important commercial types of leaf tobacco have been published by several investigators,[1] but none of them has included the comparative composition of the corresponding uncured leaf. The results of Moore and of Carpenter indicate that the cured leaf of the bright flue-cured type is quite low in ash content, and it should be added that we have had occasion to examine a number of samples grown in eastern North Carolina which contained only 6 or 7 per cent of crude ash in the leaf web. In this type the cured leaf appears to be practically free from starch, but the content of reducing sugars is quite high (16 to 18 per cent), while the content of cellulose is decidedly low, particularly in the leaf web. The leaf contains moderate quantities of nicotine, practically no nitrate, and is very low in protein, as would be expected from the light color. White Burley, which is an air-cured type, is uniformly high in ash content, according to these authors. The cured leaf appears to be free from starch and reducing sugars and is relatively high in content of cellulose. The content of nicotine is high, the content of nitrate is considerable, and the content of protein is high. It is interesting to note that Burley closely resembles the cigar-wrapper-leaf types in composition in respect to all of the above-mentioned constituents. It is also interesting to note that the peculiar chlorotic appearance of this type when growing in the field is not associated with a low content of organic nitrogenous substances. The dark fire-cured export types of Virginia, Kentucky, and Tennessee are rather high in ash content, free from starch, low in reducing sugars,[2] and somewhat low in cellulose. These types are quite high in nicotine, usually low in nitrate, and high in protein, as is to be expected from the dark color of the cured leaf. The one sample of

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[1] Moore, G. E. Report on the chemistry of American tobaccos. In Killebrew, J. B., Report on the culture and curing of tobaccos in the United States, p. 269. U. S. Department of the Interior, Census Office, Report on the Productions of Agriculture, Tenth Census, 1880. 1883.

Chemical changes in tobacco during fermentation. Connecticut Agricultural Experiment Station, 16th Annual Report, 1892, p. 29-30. 1893.

Winton, A. L., Ogden, A. W., Mitchell, W. L., and Jenkins, E. H. Effects of fertilizers on the composition of wrapper leaf tobacco. Connecticut Agricultural Experiment Station, 20th Annual Report, 1896, p. 325-326. 1897.

Carpenter, F. B. Types of tobacco and their analyses. North Carolina Agricultural Experiment Station, Bulletin 122, p. 331-366, 1895.

[2] Only two samples reported as to starch and sugars.

Maryland export leaf reported is of interest chiefly because of its unusually high content of cellulose, since this type is characterized by its "chaffiness." The analyses of cigar-wrapper leaf reported by Jenkins and Winton are fairly comparable with our results, shown in Table III, since the material was grown in the same locality and the results obtained in the two cases are not essentially different.

CHANGES IN COMPOSITION OF LEAF TOBACCO IN AIR CURING.

While the data presented in Table III show the comparative composition of cured and uncured leaf tobacco in the form there presented, they do not properly bring out the changes in composition which take place during the curing. As shown in Table I, there is a marked but variable loss of dry weight in curing, and it is evident that the simple comparison of the percentage composition of cured and uncured leaves does not give a correct index as to the actual changes in content of the various constituents involved in the curing. As pointed out by Mohr,[1] nearly all early investigators have made this error in collecting their data, with the result that many of their conclusions are entirely erroneous. There are two ways in which this difficulty can be overcome. One method is to base all comparisons on unit areas of the leaf rather than on weights, and this is undoubtedly the most satisfactory method in dealing with a small number of leaves, but where larger quantities of material are required the procedure requires much time and labor. A second method, which has been followed in our experiments, is to collect the leaves in pairs, so that each leaf which is cured shall have a duplicate which is quickly dried and weighed, as was described on page 4. In this way the total loss in weight in curing is obtained simply by comparing the weights of the cured and uncured leaves, and it is only necessary to correct the results of the analysis of the cured leaves for the total loss of weight in curing. There are necessarily some individual variations in the leaf "pairs," so that this method is not entirely reliable when only a few leaves are used, but if the composite samples include a hundred or more leaves the individual variations are of little or no consequence.

In Table IV we have corrected the results of the analyses of the cured leaf as shown in Table III for the respective loss in weight in the curing of each sample as shown in Table I, so that all results are calculated on the basis of the uncured leaf. We have added data showing the gain or loss of each constituent for the two leaf parts and for the whole leaf in each case. Table V presents in a similar manner the data for the total nitrogen and nitrogen in the various forms of combination found in the tobacco.

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[1] Mohr, E. C. J. Gepflückter und am Stamme getrockneter Tabak. Die Landwirtschaftlichen Versuchs-Stationen, Bd. 59, Heft 3/4, p. 256, 1903.

TABLE IV.—*Percentage of gain or loss of each constituent which takes place in air curing tobacco.*

[Calculated from the results shown in Tables I and III.]

Constituents.	A.—Leaves cured by the priming method.																	
	Havana Seed; 1908 (partially cured).									Halladay; 1909.								
	Leaf web.			Stems.			Whole leaf.			Leaf web.			Stems.			Whole leaf.		
	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.
Pure ash	10.97	10.73	− 0.24	17.36	16.97	− 0.39	12.72	12.43	− 0.29	15.28	16.49	+ 1.21	22.78	21.48	− 1.30	17.23	17.71	+ 0.48
Starch [1]	16.38	5.56	−10.82	10.82	8.95	− 1.87	14.88	6.39	− 8.49	5.77	4.36	− 1.41	9.94	6.91	− 3.03	6.85	5.01	− 1.84
Sugars	3.32	2.76	− .56	10.40	12.09	+ 1.69	5.27	5.34	+ .07	1.33	.55	− .78	9.78	.55	− 9.23	3.52	.58	− 2.94
Pentosans	4.21	4.30	+ .09	7.91	7.81	− .10	5.22	5.26	+ .04	4.84	4.88	+ .04	7.48	6.41	− 1.07	5.53	5.26	− .27
Cellulose (crude fiber)	8.50	8.22	− .28	13.12	13.43	+ .31	9.77	9.65	− .12	....	....	....	....	....	....	....	....	....
Oxalic acid	1.54	1.75	+ .21	1.27	1.08	− .19	1.47	1.57	+ .10	....	....	....	....	....	....	....	....	....
Citric acid	5.88	8.74	+ 2.86	3.54	2.81	− .73	5.24	7.11	+ 1.87	....	....	....	....	....	....	....	....	....
Malic acid	10.27	6.42	− 3.85	10.49	10.67	+ .18	10.33	7.59	− 2.74	....	....	....	....	....	....	....	....	....
Protein [2]	11.37	5.64	− 5.73	3.87	3.81	− .06	9.31	4.75	− 4.56	16.06	9.01	− 7.05	4.25	4.17	− .08	12.99	7.74	− 5.25
Nicotine	4.60	3.80	− .80	.51	.50	− .01	3.48	3.10	− .38	2.58	2.31	− .27	.38	.34	− .04	2.02	1.84	− .18
Nitric acid (N_2O_5)	.12	.07	− .05	1.04	1.06	+ .02	.37	.28	− .09	2.47	2.02	− .45	12.42	9.84	2.58	5.04	4.05	− .99
Ammonia	0	.14	+ .14	0	.03	+ .03	0	.12	+ .12	....	....	....	....	....	....	....	....	....
Amid and amido compounds [3]	3.22	5.13	+ 1.91	1.12	3.95	+ 2.83	2.64	4.84	+ 2.20	1.92	6.09	+ 4.17	0	1.40	+ 1.40	1.42	4.86	+ 3.44
Total determined [4]	76.18	58.95	−17.16	73.54	75.35	+ 1.81	75.51	63.17	−12.30	....	....	....	....	....	....	....	....	....

Constituents.	A.—Leaves cured by the priming method.																	
	Halladay; 1910.									John Williams broadleaf; 1911-A (artificial heat applied).								
	Leaf web.			Stems.			Whole leaf.			Leaf web.			Stems.			Whole leaf.		
	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.
Pure ash	13.03	14.04	+ 1.01	19.15	18.31	− 0.84	14.82	15.28	+ 0.46	12.16	13.29	+ 1.13	20.72	18.33	− 2.39	14.06	14.40	+ 0.34
Starch [1]	7.53	4.54	− 2.99	7.76	6.20	− 1.56	7.60	5.02	− 2.58	7.54	4.38	− 3.16	9.41	6.71	− 2.70	7.96	4.80	− 3.16
Sugars	1.97	0	− 1.97	10.88	0	−10.88	4.57	0	− 4.57	1.64	.24	− 1.40	8.40	.04	− 8.36	3.14	.20	− 2.94
Pentosans										4.72	4.74	+ .02	8.45	7.18	− 1.27	5.55	5.20	− .35
Cellulose (crude fiber)										7.82	7.94	+ .12	13.87	12.99	− .88	9.16	9.06	− .10
Oxalic acid										2.19	2.48	+ .29	1.15	.99	− .16	1.96	2.15	+ .19
Citric acid										8.52	12.28	+ 3.76	2.32	3.40	+ 1.08	7.14	10.30	+ 3.16
Malic acid										6.89	4.59	− 2.30	5.87	2.91	− 2.96	6.66	4.21	− 2.45
Protein [2]	21.20	9.95	−11.35	4.50	4.42	− .08	16.38	8.32	− 8.06	22.56	8.71	−13.85	4.63	3.72	− .91	18.56	7.60	−10.96
Nicotine	1.77	1.58	− .19	.17	.18	+ .01	1.30	1.99	− .31	3.29	3.04	− .25	.58	.59	+ .01	2.69	2.50	− .19
Nitric acid (N_2O_5)										1.07	1.37	+ .30	8.62	7.10	− 1.52	2.75	2.54	− .21
Ammonia										.07	.40	+ .33	.05	.24	+ .19	.07	.36	+ .29
Amid and amido compounds [3]										3.60	11.12	+ 7.52	0	2.42	+ 2.42	2.80	9.18	+ 6.38
Total determined [4]										77.35	69.84	− 7.49	75.62	59.44	−16.18	76.96	67.30	− 9.66

[1] Total carbohydrates hydrolyzed by dilute acid.

[2] Protein nitrogen × 6.25.

[3] Residual nitrogen × 4.8. See Table III, footnote 3.

[4] Pentosans not included.

TABLE IV.—*Percentage of gain or loss of each constituent which takes place in air curing tobacco*—Continued.

Constituents.	A.—Leaves cured by the priming method.									B.—Leaves cured on the stalk.								
	John Williams broadleaf; 1911-B (no artificial heat applied).									Havana Seed; 1908 (partially cured).								
	Leaf web.			Stems.			Whole leaf.			Leaf web.			Stems.			Whole leaf.		
	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.
Pure ash	12.02	12.97	+ 0.95	21.21	18.42	− 2.79	14.05	14.17	+ 0.12	10.36	9.48	− 0.88	17.14	15.89	− 1.25	12.22	11.25	− 0.97
Starch[1]	5.86	4.40	− 1.46	10.60	7.65	− 2.95	6.91	5.19	− 1.72	16.97	4.69	−12.28	10.64	8.50	− 2.14	15.23	5.73	− 9.50
Sugars	1.30	.22	− 1.08	7.94	.05	− 7.89	2.77	.18	− 2.59	2.29	2.13	− .16	8.57	3.53	− 5.04	4.01	2.51	− 1.50
Pentosans	4.78	4.64	− .14	9.28	7.61	− 1.67	5.77	5.30	− .47	4.45	4.16	− .29	8.04	7.62	− .42	5.44	5.11	− .33
Cellulose (crude fiber)	10.98	10.72	− .26	14.46	14.00	− .46	11.75	11.43	− .32	7.28	7.84	+ .56	13.00	13.23	+ .23	8.85	9.32	+ .47
Oxalic acid	2.32	2.48	+ .16	1.36	1.19	− .17	2.11	2.20	+ .09	1.60	1.80	+ .20	1.29	1.22	− .07	1.51	1.64	+ .13
Citric acid	9.52	13.48	+ 3.96	2.46	3.95	+ 1.49	7.96	11.37	+ 3.41	5.45	9.96	+ 4.51	4.41	4.27	− .14	5.16	8.40	+ 3.24
Malic acid	7.28	4.00	− 3.28	5.96	1.96	− 4.00	6.99	3.55	− 3.44	9.21	4.83	− 4.38	9.70	8.13	− 1.57	9.34	5.74	− 3.60
Protein[2]	21.19	8.06	−13.13	4.38	3.66	− .72	17.50	7.15	−10.35	13.12	5.20	− 7.92	3.87	3.08	− .79	10.56	4.58	− 5.98
Nicotine	3.74	3.04	− .70	.67	.63	− .04	3.06	2.60	− .46	4.11	3.73	− .38	.87	.44	− .43	3.21	2.75	− .46
Nitric acid (N_2O_5)	.87	1.38	+ .51	8.00	6.07	− 1.93	2.44	2.34	− .10	.11	.05	− .06	1.60	1.30	− .30	.52	.41	− .11
Ammonia	.03	.55	+ .52	.04	.19	+ .15	.03	.48	+ .45	.07	.05	− .02	0	.02	+ .02	.05	.05	0
Amid and amido compounds[3]	4.32	9.49	+ 5.17	0	.72	+ .72	3.37	7.74	+ 4.37	3.46	2.33	− 1.13	1.58	1.24	− .34	2.98	2.03	− .95
Total determined[4]	79.53	70.79	− 8.64	77.08	58.49	−18.59	78.94	68.42	−10.54	74.03	52.09	−21.94	72.67	60.85	−11.82	73.64	54.41	−19.23

Constituents.	B.—Leaves cured on the stalk.																	
	Halladay; 1909.									Halladay; 1910.								
	Leaf web.			Stems.			Whole leaf.			Leaf web.			Stems.			Whole leaf.		
	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.	Green.	Cured.	Gain or loss.
Pure ash	12.32	11.73	− 0.59	19.18	15.89	− 3.29	14.36	12.98	− 1.38	12.98	12.54	− 0.54	18.87	15.13	− 3.74	14.70	13.31	− 1.39
Starch [1]	8.22	5.27	− 2.95	12.41	6.57	− 5.84	9.47	5.71	− 3.76	6.14	4.85	− 1.29	8.23	5.48	− 2.75	6.75	5.01	− 1.74
Sugars	1.89	.44	− 1.45	9.77	.17	− 9.60	4.24	.36	− 3.88	1.02	0	− 1.02	8.69	0	− 8.69	3.26	0	− 3.26
Pentosans	4.61	4.58	− .03	8.54	6.21	− 2.33	5.78	5.12	− .66	....	....	....	....	....	....	....	....	....
Cellulose (crude fiber)	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Oxalic acid	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Citric acid	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Malic acid	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Protein [2]	22.44	11.15	−11.29	4.63	2.99	− 1.64	17.06	8.77	− 8.29	20.00	8.13	−11.87	4.44	3.03	− 1.41	15.44	6.97	− 8.47
Nicotine	2.17	1.60	− .57	.45	.31	− .14	1.66	1.21	− .45	1.53	1.46	− .07	.22	.12	− .10	1.15	1.07	− .08
Nitric acid (N_2O_5)	1.75	1.81	+ .06	9.92	7.19	− 2.73	4.18	3.44	− .78	1.11	1.66	+ .45	11.02	7.82	− 2.45	4.01	3.46	− .55
Ammonia	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Amid and amido compounds [3]	2.35	.73	− 1.62	0	0	0	1.65	.52	− 1.13	7.25	5.94	− 1.31	0	0	0	5.13	4.17	− .96
Total determined [4]	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....

[1] Total carbohydrates hydrolyzed by dilute acid.

[2] Protein nitrogen × 6.25.

[3] Residual nitrogen × 4.8. See Table III, footnote 3.

[4] Pentosans not included.

TABLE V.—*Percentage of gain or loss of the nitrogenous constituents which takes place in air curing tobacco.*

[Calculated from the results shown in Tables I and III.]

A.—LEAVES CURED BY THE PRIMING METHOD.

Material.	(*a*) Total nitrogen.			(*b*) Protein nitrogen.			(*c*) Nicotine nitrogen.		
	Leaf web.	Stem.	Whole leaf.	Leaf web.	Stem.	Whole leaf.	Leaf web.	Stem.	Whole leaf.
1908 tobacco—Havana Seed:									
Green material	3.32	1.22	2.75	1.82	0.62	1.49	0.80	0.09	0.61
Cured material	2.77	1.83	2.34	.90	.60	.76	.66	.09	.47
Gain or loss in curing actual	− .55	+ .61	− .41	− .92	− .02	− .73	− .14	0	− .14
percentage of total	−16.7	+50	−14.9	−50.6	....	−48.7	−17.5	0	−23
1909 tobacco—Halladay:									
Green material	4.05	3.66	3.95	2.57	.68	2.08	.44	.06	.35
Cured material	3.64	3.65	3.64	1.45	.67	1.26	.40	.07	.32
Gain or loss in curing actual	− .41	− .01	− .31	− 1.12	− .01	− .82	− .04	+ .01	− .03
percentage of total	−10.1	....	− 7.5	−43.5	....	−39.4	....	....	....
1910 tobacco—Halladay:									
Green material	4.61	2.89	4.11	3.40	.72	2.62	.31	.03	.22
Cured material	4.23	3.09	3.90	1.60	.70	1.36	.28	.03	.21
Gain or loss in curing actual	− .38	+ .20	− .21	− 1.80	− .02	− 1.26	− .03	0	− .01
percentage of total	− 8.3	+ 6.9	− 5.1	−52.9	....	−48.1	....	0	....
1911 A tobacco—John Williams broadleaf:									
Green material	5.27	3.03	4.77	3.61	.74	2.97	.57	.10	.46
Cured material	4.91	3.24	4.58	1.40	.60	1.25	.53	.11	.45
Gain or loss in curing actual	− .36	+ .21	− .19	− 2.21	− .14	− 1.72	− .04	+ .01	− .01
percentage of total	− 6.8	+ 6.9	− 4	−60.9	−19	−57.8	....	....	....
1911 B tobacco—John Williams broadleaf:									
Green material	5.18	2.84	4.66	3.39	.70	2.80	.64	.12	.53
Cured material	4.60	2.60	4.20	1.30	.59	1.16	.53	.12	.45
Gain or loss in curing actual	− .58	− .24	− .46	− 2.09	− .11	− 1.64	− .11	0	− .08
percentage of total	−11.2	− 8.5	− 9.8	−61.6	−15.9	−58.8	−17.2	0	−15.1

Material.	(*d*) Nitrate nitrogen.			(*e*) Ammonia nitrogen.			(*f*) Sum of protein, nicotine, nitrate, and ammonia nitrogen. ($b+c+d+e.$)			(*g*) Amid and amido nitrogen. ($a-f.$)		
	Leaf web.	Stem.	Whole leaf.	Leaf web.	Stem.	Whole leaf.	Leaf web.	Stem.	Whole leaf.	Leaf web.	Stem.	Whole leaf.
1908 tobacco—Havana Seed:												
Green material	0.03	0.27	0.10	0.04	0	0.03	2.69	0.96	2.23	0.63	0.26	0.52
Cured material	.02	.27	.10	.12	.02	.09	1.70	.98	1.42	1.07	.85	.92
Gain or loss in curing {actual	− .01	0	0	+ .08	+ .02	+ .06	− .99	+ .02	− .81	+ .44	+ .59	+ .40
Gain or loss in curing {percentage of total	....	0	0	+200	....	+200	−36.7	+ 2.1	−36.3	+70.0	+ 227	+77.0
1909 tobacco—Halladay:												
Green material	.64	3.22	1.31	....	....	....	3.65	3.96	3.74	.40	− [1].30	.21
Cured material	.53	2.62	1.04	....	....	....	2.38	3.36	2.62	1.26	.29	1.02
Gain or loss in curing {actual	− .11	− .60	− .27	....	....	....	− 1.27	− .60	− 1.12	+ .86	....	+ .81
Gain or loss in curing {percentage of total	−17.1	−18.6	−20.6	....	....	....	−34.2	−15.1	−29.9	+215	....	+386
1911A tobacco—John Williams broadleaf:												
Green material	.28	2.24	.72	.06	.04	.05	4.52	3.12	4.20	.75	− [1].09	.57
Cured material	.35	1.84	.63	.33	.20	.30	2.61	2.75	2.64	2.30	.49	1.99
Gain or loss in curing {actual	+ .07	− .40	− .09	+ .27	+ .16	+ .25	− 1.91	− .37	− 1.56	+ 1.55	....	+ 1.42
Gain or loss in curing {percentage of total	+25.0	−17.8	−12.5	+450	+400	+500	−42.3	−11.8	−37.1	+207	....	+249
1911B tobacco—John Williams broadleaf:												
Green material	.23	2.08	.64	.02	.03	.02	4.28	2.93	3.99	.90	− [1].09	.67
Cured material	.36	1.58	.60	.45	.16	.39	2.64	2.45	2.60	1.96	.15	1.60
Gain or loss in curing {actual	+ .13	− .50	− .04	+ .43	+ .13	+ .37	− 1.64	− .48	− 1.39	+ 1.06	....	+ .93
Gain or loss in curing {percentage of total	+56.9	−24.0	....	+2,150	+433	+1,850	−38.3	−16.4	−34.7	+118	....	+138

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[1] See Table III, footnote 3.

TABLE V.—*Percentage of gain or loss of the nitrogenous constituents which takes place in air curing tobacco*—Continued.

B.—LEAVES CURED ON THE STALK.

Material.	(a) Total nitrogen.			(b) Protein nitrogen.			(c) Nicotine nitrogen.		
	Leaf web.	Stem.	Whole leaf.	Leaf web.	Stem.	Whole leaf.	Leaf web.	Stem.	Whole leaf.
1908 tobacco—Havana Seed:									
Green material	3.61	1.51	3.03	2.10	0.62	1.69	0.71	0.15	0.55
Cured material	2.01	1.18	1.76	.83	.49	.72	.70	.08	.51
Gain or loss in curing {actual	— 1.60	— .33	— 1.27	— 1.27	— .13	— .97	— .01	— .07	— .04
Gain or loss in curing {percentage of total	—44.4	—21.8	—41.9	—60.5	—20.9	—57.4	....	—46.6	....
1909 tobacco—Halladay:									
Green material	4.91	3.03	4.34	3.59	.74	2.73	.38	.08	.29
Cured material	2.69	2.43	2.61	1.78	.49	1.39	.28	.06	.21
Gain or loss in curing {actual	— 2.22	— .60	— 1.73	— 1.81	— .25	— 1.34	— .10	— .02	— .08
Gain or loss in curing {percentage of total	—45.3	—19.8	—40	—50.4	—33.8	—49.1	—26.3	....	—27.6
1910 tobacco—Halladay:									
Green material	5.26	3.21	4.66	3.20	.71	2.47	.26	.04	.20
Cured material	3.30	2.33	3.04	1.30	.49	1.08	.23	.02	.17
Gain or loss in curing {actual	— 1.96	— .88	— 1.62	— 1.85	— .22	— 1.39	— .03	— .02	— .03
Gain or loss in curing {percentage of total	—37.0	—27.4	—34.8	—58.0	—30.9	—52.6	....	....	....

Material.	(d) Nitrate nitrogen.			(e) Ammonia nitrogen.			(f) Sum of protein, nicotine, nitrate, and ammonia nitrogen. (b+c+d+e.)			(g) Amid and amido nitrogen. (a—f.)		
	Leaf web.	Stem.	Whole leaf.	Leaf web.	Stem.	Whole leaf.	Leaf web.	Stem.	Whole leaf.	Leaf web.	Stem.	Whole leaf.
1908 tobacco—Havana Seed:												
Green material	0.03	0.41	0.13	0.05	0	0.04	2.89	1.18	2.41	0.72	0.33	0.62
Cured material	.02	.34	.12	.05	.02	.04	1.60	.93	1.39	.41	.25	.37
Gain or loss in curing {actual	— .01	— .07	— .01	0	+ .02	0	— 1.29	— .25	— 1.02	— .31	— .08	— .25
Gain or loss in curing {percentage of total	....	—17.0	....	0	....	0	—44.5	—21.2	—42.3	— .43	—24.2	—30.3
1909 tobacco—Halladay:												
Green material	.45	2.57	1.08	....	....	....	4.42	3.39	4.10	.49	— [1].36	.24
Cured material	.52	1.87	.93	....	....	....	2.58	2.42	2.53	.11	.01	.08
Gain or loss in curing {actual	+ .07	— .70	— .15	....	....	....	— 1.84	— .97	— 1.57	— .38	— .35	— .16
Gain or loss in curing {percentage of total	+15.5	—27.3	—13.9	....	....	....	—41.6	—28.5	—38.3	—77.5	— 97	—66.7
1910 tobacco—Halladay:												
Green material	.29	2.85	1.04	....	....	....	3.75	3.60	3.61	1.51	— [1].39	1.05
Cured material	.43	2.03	.87	....	....	....	1.96	2.54	2.12	1.34	— .21	.92
Gain or loss in curing {actual	+ .14	— .82	— .17	....	....	....	— 1.79	— 1.06	— 1.49	— .17	....	— .13
Gain or loss in curing {percentage of total	+48.2	—28.8	—16.3	....	....	....	—47.8	—32.2	—41.3	—11.2	....	—12.4

[1] See Table III, footnote 3.

In the experiments with picked leaves any apparent differences in the ash content of the whole leaf as between the green and cured leaves are, of course, due to slight differences in the composition of the duplicate samples and to experimental errors. As between the two leaf parts, however, it is to be observed that in every instance the leaf web appears to gain in ash content at the expense of the midrib during the curing. There is no doubt that this movement of mineral matter from stem to web takes place during the later stages of drying, and tobacco growers are familiar with the discoloration frequently caused thereby in the leaf in the vicinity of the stem. The movement is doubtless due simply to diffusion and is not to be connected with the reverse movement due to physiological translocation. As is well known, the leaf web normally dies and dries long before the stem, and the latter finally collapses rather suddenly. The remaining cell sap then oozes out into the leaf web, causing the discoloration referred to. This movement of materials, occurring after the death of the protoplasm, is not large and is localized. In the experiments in stalk curing there is a loss of ash in curing in the leaf as a whole, which, of course, can not be due to respiration and can only be accounted for by the assumption that a portion of the ash has passed into the stalk.

CHANGES IN THE CARBOHYDRATES.

In both methods of curing, nearly all the starch disappears both from the leaf web and from the midrib during the process. We have been unable to obtain a reaction for starch by the iodin test in the cured samples. This is in accordance with the results of Müller-Thurgau already referred to. The more or less complete disappearance of starch is one of the most characteristic changes involved in curing, and the relative freedom from starch of the cured leaf is a measure of the completeness of the curing process. It will be seen also that in complete curing by either method practically all of the reducing sugars disappear. The incompletely cured samples of 1908 reveal the fact, however, that the disappearance of starch precedes that of the sugars, as is to be expected, and, furthermore, that there is a temporary accumulation of sugars in the midrib, undoubtedly derived from the starch of the leaf web. It has been pointed out that analyses of flue-cured leaf have shown that under that system of rapid curing the leaf contains high percentages of reducing sugars, which are doubtless derived from the splitting up of starch, and the premature killing of the protoplasm prevents the oxidation of the sugars by respiration.

Our results as a whole indicate that the pentosans, which, generally speaking, are not physiologically plastic, undergo but little change in the leaf web, but in the stems there is a more decided decrease of these constituents. The crude-fiber content undergoes little or no change, except that in complete curing there appears to be a slight decrease in the stems, no doubt due to loss of pentosans or related hemicelluloses rather than to any change in true cellulose.

CHANGES IN THE NONVOLATILE ORGANIC ACIDS.

Tobacco leaves contain considerable quantities of oxalic, malic, and citric acids, and the changes which these undergo during the curing are interesting from the physiological point of view. That these acids are of importance in plant metabolism is generally recognized, but their rôle has not been fully determined. Because of their close relationship to the amido acids, such as aspartic acid and asparagin, it seems reasonable to associate them with protein synthesis. It is generally believed, however, that oxalic, citric, and malic acids have their origin in the incomplete oxidation of sugars when respiration proceeds in the presence of a limited oxygen supply. It appears that under certain conditions these acids may be converted partially into sugars, on the one hand, and partially into carbon dioxid and water, on the other hand. So far as we know, no distinction has been made heretofore as to the metabolic transformations which these three different acids undergo in the plant, but our results with green and cured tobacco leaves show that they behave quite differently during the curing process, which involves essentially the phenomena of respiration and of translocation.

It is evident that the content of oxalic acid is not changed during the curing, and this is equally true in both methods of curing. There is a decided loss of malic acid during the curing when the leaf is primed, as well as when it is cured on the stalk. Citric acid, on the other hand, undergoes no decrease during the curing by either method, and, indeed, there is a considerable increase in content of the acid, which is surprising in view of the behavior of the malic acid. It is, of course, conceivable that the malic acid is partially transformed into citric acid, but a simpler view is that the citric acid resists oxidation to lower products more than the malic acid, so that during the process of partial oxidation of the sugars to acids there is a gradual accumulation of citric acid, while the malic acid is more completely oxidized to water and carbon dioxid.

CHANGES IN THE NITROGENOUS CONSTITUENTS.

Referring to Tables IV and V, it will be seen that the loss in protein in curing is very large in all cases, in some instances exceeding 60 per cent of the total. The marked decrease in protein content, like the disappearance of starch, is a characteristic change involved in curing, and the relative decrease is an index of the completeness of the curing.

The decrease in starch content and in protein content furnishes a simple and reliable means of determining by chemical analysis the progress and the completeness of barn curing.

There is undoubtedly a considerable loss in nicotine content in curing due to simple volatilization, but all this loss is not shown in our experiments, for the reason that the green or uncured, as well as the cured, leaves were dried at about 80° C. and this temperature is sufficient to expel the more readily volatile portion of the nicotine.[1] In the leaf as a whole there appears to be no marked change in the content of nitric acid in either method of curing. The indicated loss of nitrate in the stem is probably only apparent, at least in part, since about the same losses are indicated for curing the primed leaves as for curing on the stalk. The determinations of nitrate were based on the well-known method of reduction with ferrous chlorid in acid solution and measurement of the oxid of nitrogen liberated. In all cases, however, it was found that with the green stems a sharp end reaction could not be obtained, for, after the principal reaction was completed, a slow evolution of gas continued almost indefinitely, apparently due to some reaction other than the reduction of the nitrate. The values for nitric acid in the green stems are therefore somewhat too high, which would, of course, have the effect of indicating a loss of nitric acid in curing. That the results for the green stems are too high is further indicated by the fact that the sum of the protein, nicotine, and nitrate nitrogen as obtained somewhat exceeds the total nitrogen in the green stems.

In the primed leaves there is a very marked increase in the amid and amido compounds, corresponding to the large decrease in protein. A considerable portion of these cleavage products of protein pass from the leaf web into the stem, which in the green condition contains practically none of these products. In the case of stalk-cured leaves there is no increase in amid and amido products, but, on the contrary, a considerable decrease, although the decrease in protein in this method of curing is even greater than in curing the primed leaves. Evidently the phenomenon of translocation is here an important factor. In the leaves cured by priming there is always a considerable loss of total nitrogen, which can not be due to translocation, and since there is no equivalent loss of nicotine the nitrogen doubtless escapes in the form of ammonia. The odor of ammonia is readily recognized in the curing barns, and there can be no doubt that it is liberated during the curing process. In other words, the cleavage products of protein are further changed, with ammonia as one of the decomposition products. The observed increase in content of ammonia, therefore, represents only a portion of the total quantity formed during the curing, the amount which becomes fixed in the leaf doubtless depending on the quantity of free acid present.

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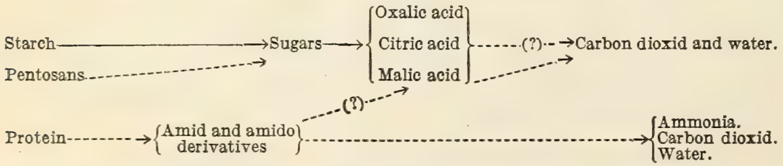
[1] Garner, W. W. Relation of nicotine to the quality of tobacco. U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin 141, p. 5–16, 1909.

Considering collectively the changes in the various constituents involved in curing, we find in the case of the primed leaves that, where the analyses are complete, the sum of the various losses recorded, less the sum of the gains, gives a total loss amounting to from 70 to 90 per cent of that obtained in Table I; or, in other words, our analyses have accounted for this proportion of the constituents which are of importance from a quantitative standpoint. A very close agreement is not to be expected, since the factors used in calculating the protein and the amid and amido constituents are probably only approximately correct. The results of the analyses account for about 75 per cent of the loss in weight indicated in Table I, where the leaves are cured on the stalk.

Aside from the observations made by Müller-Thurgau regarding the loss of starch and sugars in the curing, which have already been mentioned, the only paper bearing directly on the changes in composition during the curing, so far as known, is that of Behrens,[1] who, like nearly all previous investigators, was concerned mainly in a comparison of the methods of curing on the stalk and of curing the primed leaves. He presents data, however, showing the comparative composition of green and cured leaves. Since the total loss of weight in curing was not determined, the changes in composition can only be considered qualitatively. His analyses indicate the disappearance of starch and a decrease in sugars and protein nitrogen in curing. They show also a relative loss of total carbon and a relative gain in total nitrogen during the curing process.

While little is known as to the identity of the individual compounds making up the groups of constituents involved in the curing changes, particularly as regards the protein group and their cleavage products, the general character and course of the transformations concerned can be considered as definitely established. The following scheme shows the general course of the changes common to both methods of curing which the several groups of constituents undergo during the curing process, the prime factor in effecting these transformations being respiration, although the phenomena of translocation play a more or less important rôle, depending on the method of curing followed.

Diagram showing the general course of changes during the curing process.



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[1] Behrens, Johannes. Weitere Beiträge zur Kenntnis der Tabakpflanze. Die Landwirtschaftlichen Versuchs-Stationen, Bd. 43, p. 271-301, 18

In the above scheme the changes which take place quantitatively are indicated by solid lines, and partial transformations are indicated by broken lines. The transformation of the sugars into nonvolatile organic acids is bracketed, for the reason that, although the sugars are quantitatively removed, it is not known to what extent the acids are formed as intermediate products of oxidation. There is some question as to whether citric acid is further oxidized during the curing process, and it has not been actually proved that nitrogen-free acids are formed by the hydrolysis of the amido derivatives of protein, although this seems highly probable. The scheme merely presents the course of events in the breaking up of the surplus food supply in support of respiration, and does not take into account the movements of the soluble products due to physiological translocation.

It will be seen that the so-called ether extract, which is usually included in the analysis of agricultural products and which is intended to show the content of fat or oil, has not been considered in connection with our studies on the changes in composition taking place during the curing. It has not been considered that this determination would throw any light on the problems in hand, for the extract obtained from tobacco leaves is a hopelessly complex mixture, containing a portion of the nicotine, chlorophyll, and its decomposition products, tobacco resins, etc., and in reality containing at most very small quantities of constituents which could be properly called fat or oil. No methods are at present available for the quantitative separation of the several constituents of the ether extract, and it is obvious that the crude extract would contain several constituents already accounted for in the analyses.

The results which have been presented and discussed in the preceding paragraphs apply more particularly to typical air curing. Samples A and B of 1911, in Tables IV and V, should bring out any differences in the final result of the curing when air curing is modified by the moderate use of artificial heat. As a matter of fact these duplicate samples, after curing, show almost exactly the same composition; so that, although the moderate application of heat hastens the rate of curing, as shown by the appearance of the tobacco during the progress of the process, the final result, so far as shown by the analyses, is the same, whether or not the heat be applied. In the case of flue curing, where much higher temperatures are used, no comprehensive investigations have been made; but analyses of the cured leaves which have been reported show that the curing changes are of the same character as in air curing, the only difference being that in flue curing the transformations are less complete. In typical fire curing, in which the tobacco is only partially cured by the use of heat, the relative completeness of the chemical changes involved probably falls between that in air curing and that in flue curing.

CURING THE LEAVES ON THE STALK COMPARED WITH CURING THE PICKED LEAVES.

The relative merits of the two methods of curing have long been a subject of interest to agricultural investigators, both in this and in foreign countries, and the greater portion of the experimental work relating to tobacco curing which has been done has had special reference to this problem. Some of the earlier investigators maintained that the leaf weighed more when cured on the stalk, because of a flow of soluble material from the stalk into the leaf. Others, however, notably Nessler[1] and Behrens,[2] maintained that there was no particular difference in results as between the two methods of harvesting and curing, either with reference to weight or to quality of the cured leaf. We are indebted to Mohr[3] for the first comprehensive and decisive study of the subject. Mohr brought out clearly the errors in the methods of procedure and in the interpretation of results made by previous investigators and proved beyond doubt that for the particular type of tobacco with which he worked (cigar-wrapper leaf) the leaf cured on the stalk loses in weight approximately 11 or 12 per cent more than if cured after being picked from the stalk. This is equivalent to saying that a picked leaf after curing will weigh 11 or 12 per cent more than would the same leaf if cured on the stalk. Mohr proved conclusively by analyses of the ash and other constituents that the increased loss in weight which occurs when the leaf is cured on the stalk is due not so much to a more intense respiration but rather to a translocation of nutritive material from the leaf into the stalk.

Coming to our own experiments, by reference to Table I (p. 5) it is seen that with comparable material harvested and cured by the two methods in question during the years 1908, 1909, and 1910 the losses in dry weight by curing the leaves on the stalk were 12.2, 18.9, and 10 per cent, respectively, greater than the losses in curing the picked leaves. The larger indicated difference in loss of weight between the two methods for 1909 is to a great extent due to the unusually small loss in weight of the picked leaves, and this in turn is fully explained by the abnormally low content of starch of the picked leaves at the time of harvesting. However, the average difference between the losses in weight for the three years by the two methods of harvesting and curing is 13.7 per cent, taking the figures as they stand; and this value is in close agreement with Mohr's results for the same general type of tobacco, namely, cigar-wrapper leaf. On the other hand, Table II (p. 8) shows that the difference in loss of weight in curing between leaves harvested on the stalk and those picked from the stalk may be much greater with some of our manufacturing and export types, which are grown under different conditions and harvested in a much riper state than the cigar-wrapper types. It will be seen that in stalk curing there was a loss of about 9 to 10 per cent of the original content of pure ash, which is in close agreement with Mohr's results. In curing the primed leaves there can, of course, be no loss of ash. Our results show that, although the cured picked leaves contain little or no starch, the removal of insoluble carbohydrates is pushed considerably farther when the leaves are cured on the stalk. There is a more marked decrease of pentosans in stalk curing. There is no essential difference in the results as between the two methods with reference to sugar content, since in both cases there is a practically entire disappearance of sugars when the curing is complete.

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[1] Nessler, J. Der Tabak, seine Bestandtheile und seine Behandlung, Mannheim, 1867.

[2] Behrens, Johannes. Weitere Beiträge zur Kenntnis der Tabakpflanze. Die Landwirtschaftlichen Versuchs-Stationen, Bd. 43, p. 280, 1894.

[3] Mohr, E. C. J. Gepflückter und am Stamme getrockneter Tabak. Die Landwirtschaftlichen Versuchs-Stationen, Bd. 59, Heft 3/4, p. 253-292, 1903.

The compounds of nitrogen are decidedly the most important constituents of the leaf in regard to differences in the results of curing on the stalk as compared with curing the picked leaves. For the three years for which direct comparisons are made, the loss in protein nitrogen in the leaf web, and particularly in the midrib, was decidedly greater in stalk curing than in curing the picked leaves. Since the temperature employed in drying the samples was sufficient to expel the easily volatilized portion of the nicotine, no definite conclusion can be drawn as to the differences in loss of this constituent in the two methods of harvesting. The results regarding nitrate nitrogen are unsatisfactory, doubtless because of the difficulty in obtaining reliable results with the uncured material, as pointed out on page 28. As a whole, the results do not indicate a very marked translocation of nitrates into the stalk. As far as they were carried, our results indicate that ammonia is readily translocated into the stalk.

In comparing the results of the two methods of curing with reference to changes of composition, the most striking difference is to be found in the behavior of the nitrogenous cleavage products from the hydrolysis of protein. From 40 to 60 per cent of the protein is broken up during the curing process and, although the loss in total nitrogen in curing the picked leaves shows conclusively that a portion of the nitrogen of the decomposition products escapes as ammonia, the loss thus involved is but a comparatively small portion of the whole. The result is that there is a marked accumulation of amid and amido compounds in the cured picked leaves, the increase in this form of nitrogen amounting to 0.5 to 1.5 per cent of the leaf weight, or 100 to 400 per cent more than was contained in the uncured leaf. In the stalk-cured leaves, on the other hand, not only are the products resulting from the splitting of the protein completely removed, but a considerable portion of the amid and amido compounds originally present in the leaf are also removed in the process of curing. In stalk curing, the loss of nitrogen in these forms amounts to 35 to 45 per cent of the total protein and amid and amido nitrogen, while in curing the picked leaves the direct loss of nitrogen in these forms by the splitting off and escape of ammonia averages less than 10 per cent of the total. In stalk curing, the content of amid and amido nitrogen alone is reduced by about 15 to 60 per cent, depending on the original content of these forms of nitrogen. As regards total nitrogen, the loss in curing the picked leaves is from 4 to 15 per cent of the total, while the loss in stalk curing is from 35 to 42 per cent.

As regards the organic acids, there is no decided difference in the behavior of oxalic acid in the two methods of curing, and this is true also as to malic acid. Citric acid seems to accumulate to approximately the same extent whether the leaf is attached to the stalk or is detached during the curing. There is no satisfactory evidence that these acids undergo translocation in stalk curing.

Summing up the more important differences in the results of curing the leaves on the stalk as compared with curing the picked leaves which are brought out in our experiments, it is seen that the loss in weight of dry matter is 10 to 15 per cent greater when the leaves are cured on the stalk. This difference is due mainly to the more complete removal of carbohydrates, including the pentosans, and of protein and, more particularly, to the translocation of the cleavage products of protein and of the mineral constituents from the leaf into the stalk. In stalk curing, in addition to the respiration activities which constitute the important factor in curing the picked leaves, the translocation of mobile materials from the leaf into the stalk assumes an important rôle. This translocation of materials from leaf to stalk in stalk curing constitutes, in fact, the essential difference between this method of curing and that in use after the leaves are removed from the stalk. Our results on this phase of tobacco curing agree closely with the conclusions reached by Mohr from his study of the subject. These results find additional support in the work of Johnson [1] relating to the composition of two lots of tobacco stalks, from one of which the leaves were removed at the time of harvesting, while the second lot was allowed to cure with the leaves attached. The analyses of the two lots of stalks show that those which were allowed to cure with the leaves attached gained approximately 30 per cent of their original content of total nitrogen, 36 per cent of their content of phosphoric acid, and 8 per cent of their content of potash. This gain in nitrogen, phosphoric acid, and potash can only be accounted for on the assumption that these constituents were transported from the leaf into the stalk during the process of curing. It is evident that the phenomena of translocation in the curing of tobacco leaves attached to the stalk follow essentially the same laws as obtain during the growth and development of plants under normal conditions. The movement of reserve nutrients is from the leaf web through the veins and midrib into the stalk and normally thence to the younger growing parts. Only such constituents as are the most essential, physiologically, undergo translocation to a marked degree.

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[1] Johnson, S. W. Analyses of tobacco stalks when cut and after curing. Connecticut Agricultural Experiment Station, 16th Annual Report, 1892, p. 31–34. 1893.

ENZYMS IN TOBACCO CURING.

It has been pointed out in the preceding pages that tobacco curing consists, primarily, in the hydrolysis of insoluble carbohydrates and proteins, followed by a partial or complete removal of the cleavage products by further hydrolysis and by oxidation and, in stalk curing, by translocation into the stalk. It is quite generally recognized that the immediate agencies effecting transformations of this character in the vital activities of the plant are enzyms. We have shown that starch and pentosans are converted into reducing sugars, which are then oxidized or translocated, and that protein is converted into the simpler amid or amido derivatives, from which, in turn, ammonia is split off. It seems probable, therefore, that diastases, cytases, and proteolytic and deamidizing enzyms take part in the curing of the tobacco leaf. It seems very probable also that the oxidases play an important part, particularly as regards the changes in color which the leaf undergoes in curing.

We have not attempted to go into a study of the enzyms concerned in tobacco curing, except simply to bring out the fact that their activities are for the most part intimately associated with and dependent upon the presence of the living protoplasm. We have already pointed out that a ripe tobacco leaf will not cure properly, even under the most favorable conditions, if it has been previously subjected to very high or low temperatures or to the action of protoplasmic poisons. It has also been stated that the progress of the curing can be readily followed by determining at different stages the relative quantities of unchanged starch and protein contained in the leaf. The following experiments show by chemical methods that the premature killing of the protoplasm prevents the changes in composition which are essential to successful curing.

In the first place, an experiment was carried out to determine the effectiveness of rapid drying at high temperatures in preventing these changes in composition. Two lots of leaves, containing 14 leaves in each lot, were selected in the manner described on page 4, and one of these lots was allowed to cure normally. The midribs were removed from the second lot of leaves and the half of each leaf thus resulting was plunged into boiling absolute alcohol for a few minutes, while the second set of leaf halves was immediately placed in an air bath heated to 90° C. and dried. The results of the experiment are shown in Table VI. In the case of material killed with alcohol the results were corrected, of course, for the matter extracted by the alcohol. To facilitate comparison, the results are presented on a uniform basis of 14 whole leaves, exclusive of the midribs.

TABLE VI.—*Effectiveness of killing fresh tobacco leaves at high temperatures, as compared with killing with hot alcohol in arresting changes in composition.*

1911 material.	(*a*) Leaf web killed with alcohol.	(*b*) Leaf web killed at high temperature.	(*c*) Leaf cured normally.
Dry weight of 14 leaves........grams..	126.2	124.6	97.5
Loss of weight in curing........per cent..	....	1.3	22.8
Starch........do....	26.64	24.57	5.17
Protein nitrogen........do....	2.04	2.13	1.22
Reducing sugars........do....	1.21	1.63	3.86

In the above table the results are calculated on a basis of the original dry weight of the leaves, as represented by *a*. These results show that, while subjecting the leaf to a temperature of 90° C. does not immediately stop all action upon the carbohydrates, this method, which was employed in all the experiments already described, answers satisfactorily for the purposes in view. Having established this point, the effect on the curing process of chloroforming the leaf was next studied. A lot of five ripe leaves was collected and their midribs removed. The one lot of leaf halves thus obtained was immediately dried at 90° C., as in the preceding experiment, while the second lot was exposed to chloroform vapors in a closed jar for a few minutes and then placed under the proper conditions for normal curing. After four days this material was also dried at 90° C. The results of the experiment are shown in Table VII.

TABLE VII.—*Effect of exposure to chloroform on the curing of tobacco leaves.*

1912 material.	Leaf dried at 90° C.	Leaf chloroformed and then "cured."
Dry weight of 5 half leaves........grams..	18.7	19.00
Starch........per cent..	41.74	43.88
Protein nitrogen........do....	1.17	1.23

It is obvious that exposure to chloroform effectually prevents those changes in composition which are characteristic of normal curing and, indeed, the appearance and properties of leaves so treated clearly show that normal curing does not take place. Since these normal changes in composition are effected by hydrolytic enzyms, and since it is well known that the green leaves of plants normally contain these enzyms, it is not clear why protoplasmic poisons which do not inhibit the action of such enzyms should prevent the progress of the curing along these lines. Brown and Morris [1] considered this matter in connection with their investigations on the occurrence of diastase in the leaves of plants, but did not arrive at a satisfactory conclusion. They found that, although the diastatic activity of the leaf increases markedly when it is kept in darkness, even when a leaf has been subjected to this treatment there is no further decrease of starch after treatment with chloroform. Whatever the explanation of this phenomenon, it seems logical, as suggested by Brown and Morris, that partial starvation will lead to an increased formation by the leaf cell of enzyms designed to furnish the needed nourishment. We have found that such is the case as regards diastase in the curing of tobacco leaves. The method used for comparing the diastatic activity of fresh and partially cured leaves was that described by the above-named investigators, and consists essentially in digesting a given weight of the material with a 2 per cent water solution of soluble starch under proper conditions and with suitable controls. The increase in reducing sugars is taken as a measure of the relative diastatic activity.

In the following experiments, after having obtained the leaf areas, one half of each leaf was chloroformed and dried at 35° C., while the remaining half of each leaf was maintained under normal curing conditions (slow drying in darkness and at moderate temperatures) for a period of 43 hours in experiment 1 and for one week in experiment 2.

The material chosen for the first experiment was a single mature bottom leaf taken from a Connecticut Broadleaf plant grown in the greenhouse, and in the second experiment one bottom and two top leaves were taken from a mature plant of Yellow Pryor grown under normal field conditions. The cured material was chloroformed and dried in the same manner as the uncured. The diastatic activity was determined for equal areas and not equal weights of the cured and uncured material, and the results as reported are in terms of the weights of maltose formed by digesting 10 grams of the uncured samples chosen as standards and weights corresponding to an equal area of the other samples with 2 per cent soluble starch solution for 48 hours at 30° C. It is necessary to use equal areas of leaf to correct properly for the loss in dry weight in curing. The results of these experiments are shown in Table VIII.

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[1] Brown, H. T., and Morris, G. H. Contribution to the chemistry and physiology of foliage leaves. Journal, Chemical Society [London], Transactions, v. 63, p. 604-659, 1893.

TABLE VIII.—*Diastatic activity of uncured and partially cured tobacco leaves.*

Material.	Total weight of material.	Weight per square foot.	Loss of weight in curing.	Weight of leaf used.	Relative diastatic activity.
Experiment 1 (14 square inches of leaf surface used):					
A bottom leaf—	*Grams.*	*Grams.*	*Per cent.*	*Grams.*	
Green half	6.47	5.124		0.5000	4.41
Cured half	5.67	4.489	12.4	.4378	5.48
Experiment 2 (9.8 square inches of leaf surface used):					
2 top leaves—					
Green halves	7.634	7.354		.5000	2.04
Cured halves	4.836	4.659	36.7	.3167	7.02
A bottom leaf—					
Green half	8.536	7.940		.5398	2.48
Cured half	5.004	4.655	41.4	.3164	6.50

The above results show very clearly that there is a marked increase in the diastatic activity of tobacco leaves during the curing process. It seems probable that there is a similar increase in the proteolytic enzyms during the curing, but no attempt was made to determine this point.

TOBACCO CURING AS AFFECTED BY EXTERNAL CONDITIONS.

Since respiration plays a fundamental rôle in tobacco curing, it is to be expected that the external conditions, notably the temperature, will have a decided influence on the curing process. The factors of importance in this connection are the temperature and relative content of moisture, oxygen, and carbon dioxid of the surrounding atmosphere and the presence or absence of light. We have made some experiments on the relation of the first two of these factors to the rate of curing, using the quantities of starch and protein hydrolyzed as a measure of the progress of the curing.

EFFECT OF TEMPERATURE ON RATE OF CURING.

In 1911 three lots of six leaves each were collected, using the precautions already described in detail, to have the three lots strictly comparable. The stems were removed from the first lot of leaves (*a*) and the leaf web killed by plunging into boiling absolute alcohol, after which the leaf web and stems were dried at 80° C. The second lot of leaves (*b*) was placed in a bell jar and maintained at a temperature of approximately 10° C. (50° F.) for 24 hours, after which they were treated exactly like the first lot. The third lot (*c*) was similarly maintained at a temperature of approximately 24° C. (75° F.) for 24 hours and then treated like the other two lots. In 1912 the experiment was repeated with two lots of five leaves each. The stems were removed from the first lot (*a*), one half of each leaf being quickly dried at 90° C., while the remaining leaf halves were chloroformed and placed under favorable conditions for curing for a period of four days before being dried in the oven. (This material served also for the data presented in Table VII.) The stems were also removed from the second lot of leaves (*b*) and one half of each leaf maintained in a bell jar at an average temperature of 12° C. (54° F.) for 48 hours, while the second half of each leaf was similarly treated, but at an average temperature of 27° C. (80° F.). These leaf halves were then quickly dried at 90° C. The results of these experiments are given in Table IX.

TABLE IX.—*Effect of temperature in hastening rate of curing.*

Material.	(*a*) Leaf killed with alcohol (1911) or with heat (1912).	(*b*) Leaf partially cured at 10° to 12° C.	(*c*) Leaf partially cured at 24° to 27° C.
1911 samples:			
Total dry weight of 6 leaves, including stems..........grams..	61.1	61.5	57.6
Starch (leaf web)..........per cent..	28.47	25.17	21.33
Protein nitrogen (leaf web)..........do....	1.90	1.94	1.59
1912 samples:			
Total dry weight of 5 half leaves, without stems..........grams..	18.7	18.3	17.4
Starch..........per cent..	41.74	37	26.46
Protein nitrogen..........do....	1.17	1.03	.87

From the above table it is seen that where the leaf was allowed to cure for 24 hours there was a loss of about 3.3 per cent of the starch content at the lower temperature and of 7.1 per cent at the higher temperature, while for the leaf tissue cured for 48 hours the losses were 4.7 per cent and 15.3 per cent, respectively. There was no decrease in protein in the first case at the lower temperature, but a considerable decrease at the higher temperature; and in the second case there was only a small decrease at the lower temperature, but the decrease at the higher temperature was very marked. It is evident that the rate of curing is proportional to the temperature, which is in accord with the well-known principle that normal respiration, as measured by the rate of evolution of carbon dioxid, increases regularly with rise in temperature; and it is interesting to note that in tobacco curing the rate of increase in the speed of the reactions involved tends to obey the law of Van't Hoff that the speed of ordinary chemical reactions is approximately doubled for each increase of 10 degrees centigrade in temperature, provided, of course, that in this case the optimum temperature for the enzyms involved in curing is not much exceeded.

EFFECT OF MOISTURE ON THE RATE OF CURING.

It is well known that the rate of drying is of fundamental importance in practical tobacco curing, and when the quantity of water in the leaf has been reduced to certain limits the progress of the curing is practically stopped. On the other hand, if too much moisture remains in the leaf toward the end of the curing, when the leaf cells are dying, abnormal processes of decay, commonly known as pole-sweat, set in, which result in the disintegration of the leaf tissue. This latter trouble is due, however, to the invasion of foreign organisms and needs no further consideration here. Aside from these extreme conditions, we have to consider the effects of partial loss of water by the leaf in the course of the curing process. It is stated by some authorities that normal respiration is most active when the cells are fully turgid; that is, in the presence of a maximum amount of water. It appears from our experiments, however, that this principle does not hold so far as it relates to tobacco curing.

In 1911 two ripe tobacco leaves were selected and the midribs removed. The two right-hand leaf halves were suspended in a tall closed jar for 24 hours over concentrated sulphuric acid on which was floated a dish containing a little caustic soda. The corresponding left-hand leaf halves were similarly placed in a jar containing only the caustic soda. The two lots of material were then killed with hot alcohol in the usual manner and prepared for analysis. The material which had stood over sulphuric acid was quite limp from loss of water, but the other sample had scarcely wilted. The leaves used in this experiment are comparable with those used for the 1911 experiment shown in Table IX and therefore originally contained approximately 28.5 per cent of starch and 1.9 per cent of protein nitrogen. A similar experiment was made in 1912 with a lot of five leaves, the corresponding halves of which were subjected to the different rates of drying for 28 hours and then quickly dried at 90° C. This material is comparable with the 1912 samples used in the experiment presented in Table IX and therefore originally contained approximately 42 per cent of starch and 1.2 per cent of protein nitrogen. The results of the tests are shown in Table X.

TABLE X.—*Effect of partial drying on rate of curing.*

Material.	Leaf partially dried.	Leaf not dried.
1911 samples:		
Total dry weight of 2 leaf halves....grams..	8.7	8.7
Starch....per cent..	12.61	18.27
Protein nitrogen....do....	1.49	1.56
1912 samples:		
Total dry weight of 5 leaf halves....grams..	19.05	19.75
Starch....per cent..	30.76	34.55
Protein nitrogen....do....	1.13	1.10

The final dry weight of the two 1911 samples was the same, but as there was a marked difference in starch content at the end of the experiment it is evident that the leaf halves subjected to partial drying were originally somewhat heavier than those not subjected to drying. It is seen that initial partial drying materially hastens the disappearance of starch, but for the interval of time covered by the experiments there was no important difference in the rate of hydrolysis of protein. The less-marked differences as regards starch in the 1912 experiment were doubtless due to the fact that there was much less difference in water content (about 8 per cent) between the two samples as a result of the partial drying than was the case with the 1911 samples. In view of these results it appears that the thorough wilting of the tobacco leaves at the beginning of the curing process promotes good curing.

SUMMARY.

Curing is essentially a life process, as is shown by the fact that killing the protoplasm at excessively low or high temperatures or by means of poisons, such as chloroform, effectually prevents normal curing. It follows, also, that imperfect curing can not be fully corrected in the subsequent process of fermentation, which is not dependent on the living protoplasm.

Experiments covering a period of four years have shown that in the case of cigar-wrapper-leaf types the average loss in weight of dry matter in curing the picked leaves is 12 to 15 per cent, while in curing the leaves on the stalk the loss in dry weight is approximately twice as great. In other words, a cigar-wrapper leaf picked from the stalk will weigh after curing approximately 14 to 18 per cent more than would the same leaf when cured on the stalk.

In the curing of the export and manufacturing types and of cigar-filler types, which are harvested in a riper or more mature condition, the loss in weight of dry matter is greater than in the case of cigar-wrapper leaf, frequently amounting to 35 to 40 per cent, even when the leaves are picked from the stalk in harvesting. With most of the export and manufacturing types the stalks are split in harvesting, and under these conditions the loss in dry matter in curing is considerably less than when the stalk is simply severed near the base in harvesting.

The chemical changes which take place in curing can only be properly brought out by presenting all results on the basis of the uncured leaf. It has been shown that in thorough air curing all starch and reducing sugars disappear and there is a decrease of pentosans and malic acid, while there is an increase in citric acid and the cellulose content remains unchanged. There is a large decrease in protein, in some cases amounting to 60 per cent of the total, and a considerable decrease in nicotine and total nitrogen. Appreciable quantities of ammonia are formed in the process.

In the curing of picked leaves the chemical changes appear to be due almost wholly to respiration, while in curing the leaves on the stalk the phenomenon of translocation from the leaf into the stalk

plays an important rôle. This translocation, which constitutes the essential physiological difference in the two methods of curing, involves the transfer into the stalk of the amid and amido compounds derived from the protein, ammonia and a portion of the mineral constituents, nitrate and, doubtless, a portion of the carbohydrates. The picked leaves after curing contain, therefore, much larger quantities of amid and amido compounds, and ammonia and somewhat larger quantities of mineral matter and nitrate than the leaves cured on the stalk.

The physiological processes characteristic of tobacco curing indicate the presence of diastatic, proteolytic, and deamidizing enzyms, and probably also of oxidases. The process of starvation to which the leaves are subjected leads to an increased secretion of diastase during the progress of the curing.

Temperature has a very marked effect on the rate of curing. The rate of curing increases very rapidly with rise in temperature up to the killing point of the protoplasm (about 130° F.). The moderate use of artificial heat in air curing does not materially affect the final result in curing so far as measured by the ordinary methods of chemical analysis, provided other conditions remain favorable in both cases.

Thorough wilting in the initial stages of the curing promotes the progress of the process, provided the further drying of the leaf is not allowed to proceed too rapidly.

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